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TITLE: Integrated monolithic microfabricated electrospray and liquid
chromatography system and method
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PARENT-CASE:
REFERENCE TO RELATED APPLICATIONS This application is related to copending U.S.
application Ser. No. 09/156,507, entitled INTEGRATED MONOLITHIC
MICROFABRICATED ELECTROSPRAY AND LIQUID CHROMATOGRAPHY SYSTEM AND METHOD, filed
Sep. 17, 1998.

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DWKU:
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BSPR:
Liquid chromatography (LC) is a well-established analytical method for
separating components of a fluid for subsequent analysis and/or identification.
Traditionally, liquid chromatography utilizes a separation column, such as a
cylindrical tube, filled with tightly packed beads, gel or other appropriate
particulate material to provide a large surface area. The large surface area
facilitates fluid interactions with the particulate material, and the tightly
packed, random spacing of the particulate material forces the liquid to travel
over a much longer effective path than the length of the column. In
particular, the components of the fluid interact with the **stationary phase** (the

particles in the liquid chromatography column) as well as the mobile phase (the liquid eluent flowing through the liquid chromatography column) based on the partition coefficients for each of the components. The partition coefficient is a defined as the ratio of the time an analyte spends interacting with the **stationary phase** to the time spent interacting with the mobile phase. The longer an analyte interacts with the **stationary phase,** the higher the partition coefficient and the longer the analyte is retained on the liquid chromatography column. The components may be detected spectroscopically after elution from the liquid chromatography column by coupling the exit of the column to a post-column detector.

BSPR:
Capillary electrochromatography is a hybrid technique which utilizes the electrically driven flow characteristics of electrophoretic separation methods within capillary columns packed with a solid **stationary phase** typical of liquid chromatography. It couples the separation power of reversed-phase liquid chromatography with the high efficiencies of capillary electrophoresis. Higher efficiencies are obtainable for capillary electrochromatography separations over liquid chromatography because the flow profile resulting from electroosmotic flow is flat due to the reduction in frictional drag along the walls of the separation channel when compared to the parabolic flow profile resulting from pressure driven flows. Furthermore, smaller particle sizes can be used in capillary electrochromatography than in liquid chromatography because no back pressure is generated by electroosmotic flow. In contrast to electrophoresis, capillary electrochromatography is capable of separating neutral molecules due to analyte partitioning between the

stationary and mobile
phases of the column particles using a liquid
chromatography separation
mechanism.

DEPR:
The plurality of separation posts 416 extends from a side wall of the
separation channel 412 in a direction perpendicular to the fluid flow though
the separation channel 412. Preferably, one of the ends of each separation
post 416 does not extend beyond and is preferably coplanar or level with the
second surface 417. The separation channel 412 is functionally similar to the
liquid chromatography column in that component separation occurs in the
separation channel 412 where the plurality of separation posts 416 perform the
liquid chromatography function. Component separation occurs through the
interaction of the fluid flowing through the separation channel 412 wherein the
columnar separation posts 416 provides the large surface area. The surfaces of
the separation channel 412 and the separation posts 416 are preferably provided
with an insulating layer to insulate the fluid in the separation channel 412
from the substrate 402. Specifically, the separation posts 416 are preferably
oxidized silicon posts which may be chemically modified using known techniques
in order to optimize the interaction of the components of the sample fluid with
the **stationary phase,** the separation posts 416. In one embodiment, the
separation channel 412 extends beyond the separation posts 416 to the edge of
the substrate 402 and terminating as the exit orifice 414.

DEPR:
Further, all features such as the reservoir, the separation channel and the
separation posts are recessed from the substrate 402. The portion of the
substrate 402 exterior to the reservoir and the separation

channel thus serves
to physically protect the separation posts from casual abrasion and stress
fracture in handling and subsequent bonding of the substrate 402 and the cover
420. Because the posts are integral with the substrate, the posts are
inherently stable and thus allow for the use of a pressurized system without
the risk of damage to the **stationary phase** which may otherwise result with the
use of conventional packing materials in conventional high-performance liquid
chromatography systems.

DEPR:
After the fluid samples pass the SPE posts 416L, waste products from, for
example, the solid-phase extraction process may be directed into a waste
reservoir 410Q. In particular, during the SPE process, voltage differences may
be applied between or amongst reservoirs 410M, 410N, 410P, and 410Q such that a
portion of the fluid from reservoirs 410M, 410N is directed to waste reservoir
410Q while the remaining portion of the fluid from reservoir 410M remain on the
SPE posts 416L. Material may then be washed off of the SPE posts 416L by
directing fluid from, for example, reservoir 410P through channel 412M for
separation of the extracted material by separation posts 416M. Additional
reservoirs 410R, 410S downstream of the waste reservoir 410Q and upstream of
the separation posts 416M may be provided to contain gradient elution of
analytes in one reservoir and a diluent in the other reservoir. Gradient
elution facilitates chromatography by changing the mobile phase composition,
i.e. the polarity to facilitate analyte interactions with the **stationary phase,**
and thus facilitate separation of the analytes. In addition, the diluent
provides the correct polarity of the solution for the next separation.